SEXUAL PHASES IN TERRESTRIAL NEMERTEANS

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There has been much confusion in the literature regarding the sexuality of the various species of terrestrial nemerteans, due partly to the small number of individuals that have been available for study and partly to the fact that in some cases only the older and larger individuals have been collected. Since some of the species have a distinct tendency toward protandry and some are irregularly hermaphroditic, the successive stages in the life history are necessary in order to determine the potential sexuality of the individual.

Particularly confusing have been the accounts of the sexual conditions in *Geonemertes palaensis,* a species found on Pelew (Palao), Samoa and several other tropical Pacific islands. A collection consisting of 21 representatives of this species recently submitted to the writer for study included individuals representing such a series of sexual phases as will give a presumably reliable clue to their sequence. This occasion has also been taken to re-examine the consecutive sexual phases of the Bermuda land nemertean (*G. agricola*) as described many years ago (Coe, 1904).

The 12 apparently valid species of terrestrial nemerteans which have been described up to the present time all belong to the genus *Geonemertes.* Three of these, *G. australiensis* Dendy, *G. hillii* Hett, and *G. rodericana* (Gull.) are described as of separate sexes and oviparous, with the males usually smaller than the females. Three others, *G. dendyi* Dakin, *G. graffi* Bürger and *G. novae-zelandiae* Dendy have been thought to be of separate sexes, although no males have yet been found. One species, *G. spirospermia* Darbishire, is known only from a single male and another, *G. caeca* Darbishire, from a single female. *G. chalicophora* Graff was first described as hermaphroditic, although Böhmig (1898) found only females. *G. palaensis* Semper is hermaphroditic, as originally described and as found by von Kennel (1878), but only ovaries were present in the single specimen studied by Schröder (1918) as well as in the five investigated by Hett (1928). *G. arboricola* Pun-

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nett is likewise hermaphroditic and, as pointed out by Hett (1928), resembles *G. palaensis* so closely as to suggest specific identity.

All the above are known or assumed to be oviparous but the land nemertean of Bermuda, *G. agricola* (Will. Suhm), has been shown to be irregularly protandric, hermaphroditic and viviparous (Coe, 1904). The egg clusters of *G. australiensis* have been described by Dendy (1893) and those of *G. dendyi* by Waterston and Quick (1937). Also one of the specimens of *G. palaensis* studied by Hett (1928) had an egg cluster attached to the proboscis.

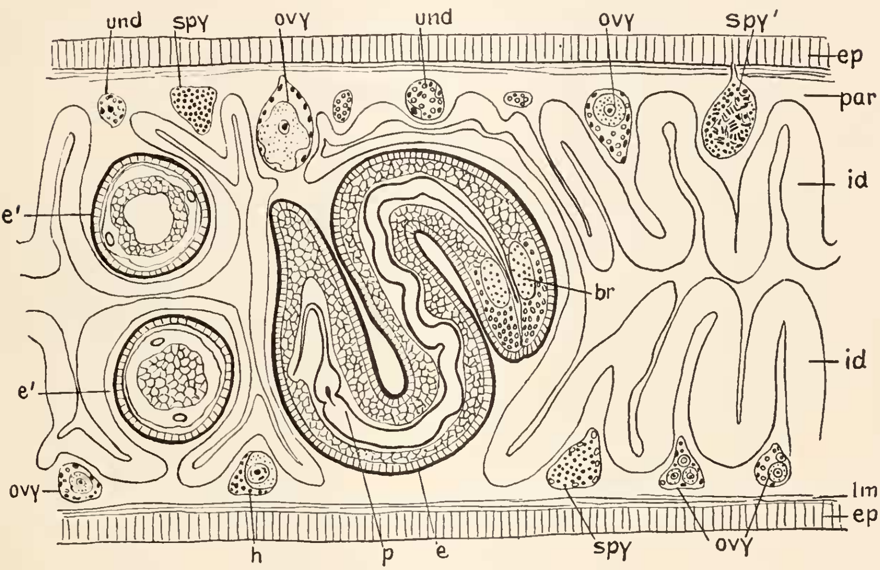

FIG. 1. *Geonemertes agricola.* Portion of horizontal section of body dorsal to nerve cords, showing sections of two embryos (*e, e'*) nearly ready for birth. Small gonads representing young ovaries (*ovy*), young spermaries (*spy*), spermaries with ripe spermatozoa (*spy'*), gonads of the hermaphroditic type (*h*), as well as others still remaining undifferentiated (*und*) are already present in anticipation of the following sexual phases. Other letters represent: *br*, brain of embryo; *ep*, epithelium of body wall; *id*, intestinal diverticula; *lm*, longitudinal musculature of body wall; *p*, proboscis of embryo, *par*, parenchyma. Slightly diagrammatic.

Sexual Phases in G. agricola

The study of more than a hundred individuals representing all ages of *G. agricola* shows that the young worms are usually protandric, since at first sexual maturity they have their bodies distended with spermaries. After the discharge of the sperm or sometimes earlier, ovaries begin to develop and the ovocytes slowly grow to maturity in the first female phase. Before the eggs are ready for fertilization, however, other gonads in the same individual may develop rapidly into spermaries, and some of these may form functional spermatozoa in time to fertilize the eggs of the same individual or of another individual which may be in a similar female phase.

Shortly after fertilization the oviducts are closed and the eggs undergo development. In some individuals only a few embryos are formed (Fig. 1) but in others the numbers are so great that the mother's body

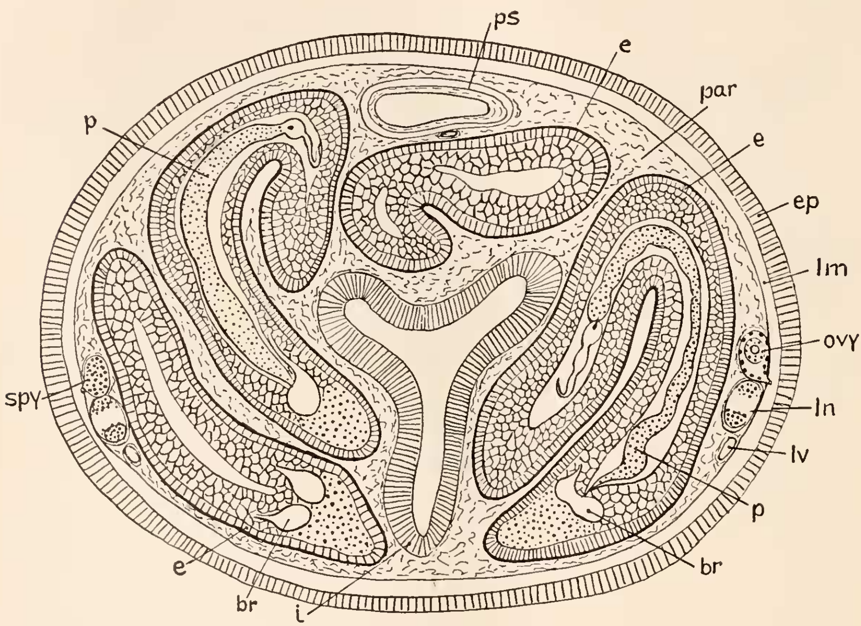

FIG. 2. *Geonemertes agricola.* Transverse section of body distended with embryos of which four have been cut in this section; showing also small ovary (*ovy*) and small spermary (*spy*); *i*, intestine; *ps*, proboscis sheath; other letters as in Figure 1. Slightly diagrammatic.

becomes enormously distended (Fig. 2). After all the organ systems of the adult except the reproductive organs have been acquired the young worm forces its way out of the mother's body by rupturing her body walls.

Even while embryos remain in the body, preparations are in progress for the succeeding sexual phases, both spermaries and ovaries continuing to be differentiated (Fig. 1).

After the discharge of the embryos such spermaries as have not previously been active now become functional as the second male phase.

This is later followed by the second female phase, with some new spermaries becoming functional when the ova are fully ripe.

The primary male phase is thus followed by a female phase which often changes to a functional hermaphroditic phase before the ova are ready for fertilization. Then follows a series of male and female (or hermaphroditic) phases, probably with seasonal interruptions of considerable duration, throughout the lifetime of the individual. There is much individual variation, however, in regard to the relative preponderance of male and female characteristics and time of maturity of each type of sexual products, since some individuals in the female or hermaphroditic phase have many large ovaries and few spermaries; others have

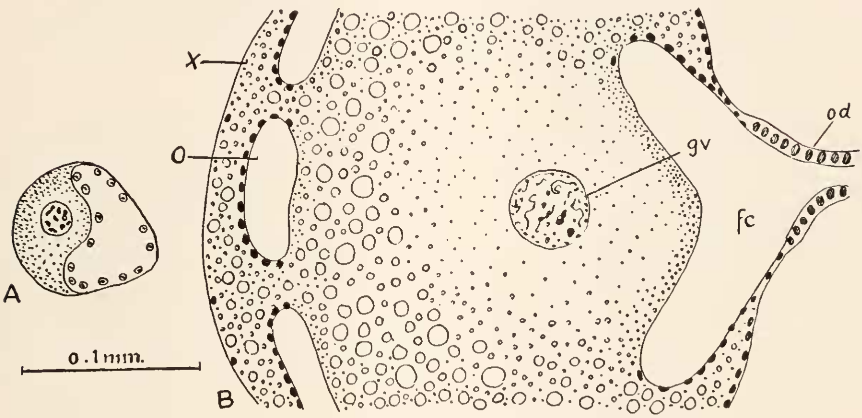

FIG. 3. *Geonemertes agricola*. *A*, young ovary with single ovocyte and associated follicle cells lining ovarian cavity. *B*, nearly mature ovary, showing portion of the single ovum with part of original ovarian cavity converted into a fertilization chamber (*fc*) continuous with the oviduct (*od*); *x*, peripheral cytoplasm; *o*, extensions of ovarian cavity lined peripherally with yolk-forming follicle cells; *gv*, germinal vesicle surrounded by yolk-free cytoplasm. Scale indicates magnification.

a true female phase with no spermaries at the time. Variations in the proportion of primary gonads which are to become differentiated into the two types of sexual glands and differences in the time of their development would account for the diversity of sexual conditions observed.

Differentiation of Gonads

It has been previously reported (Coe, 1904) that the gonads, all of which are primarily ambisexual in nature, become differentiated into three types before maturity. In those which are to form ovaries the ovocyte increases rapidly in size, first by assimilating smaller potential ovocytes and spermatogenic cells (if present) and later by yolk-formation through the activities of the follicle cells in the peripheral layer of cytoplasm (Fig. 3) as described more fully in the account of ovogenesis in *G. palaensis* in the following section of this report. In the spermaries the potential ovocytes are inhibited in their growth by the multiplication of spermatogonia.

Relatively few of the gonads retain their primary ambisexual character to maturity by the harmonious development of both types of gametes. These few hermaphroditic gonads apparently discharge their spermatozoa previous to yolk-formation by the ovocyte. In other cases the ovocyte in such gonads undergoes fragmentation and cytolysis following the discharge of the sperm.

The ripe ova are from 0.35–0.45 mm. in diameter, or from one-fourth to one-half as great as the diameter of the entire body of the worm. Development is of the direct type (Coe, 1904).

Sexual Phases in G. palaensis

This species appears to be normally oviparous as evidenced by the finding of an egg cluster by Hett (1928). The material now available for study shows that the sexual phases are similar to those reported previously for *G. agricola* (Coe, 1904) and summarized above. The primary functional male phase is followed similarly by successive female (potentially hermaphroditic) and male phases, the extent of hermaphroditism depending on the time of development of the spermaries and their relative number.

Of the 21 specimens in this collection 2 were in the functional male phase at the time of collection, with ripe spermaries and immature ovaries; 3 were functionally female at the time of collection, with ovaries showing various stages of ovogenesis but no spermaries; 8 were unripe sexually with small ovaries and undeveloped spermaries; and 8 were functional hermaphrodites with large ova and ripe or nearly ripe spermaries.

The potentially hermaphroditic phases may in certain individuals or at certain periods in the sexual sequence be predominantly male or predominantly female or exclusively functional as one sex or the other. A greater number of individuals will, however, be found in the female phase than in the male phase of sexuality, since the time required for the development of the ovaries is much greater than for the spermaries. Consequently, if the collectors had obtained only such of these specimens as were in the exclusively female phase at the time of collection, it would have been concluded that the species must be of separate sexes. It is suspected that this may be the case with some of the other species which are known from only one or a few specimens and in which neither protandry nor hermaphroditism has been observed.

The males or the primary male-phase individuals, whichever they may be, are smaller than the functional females and consequently less

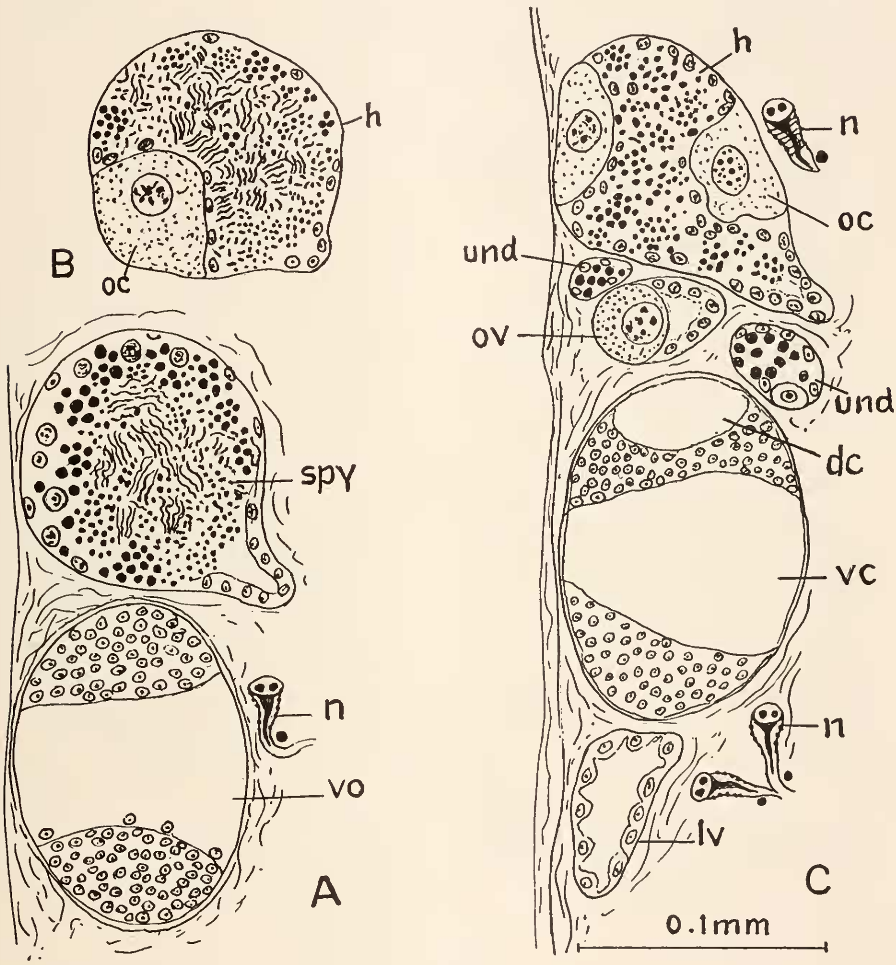

Fig. 4. *Geonemertes agricola.* *A,* transverse section of lateral nerve cord (*vo*) and adjacent spermary (*spy*) from individual containing fully grown embryos; *n,* a single flame cell of nephridial system. *B,* hermaphroditic gonad with single ovocyte (*oc*) from same individual. *C, Geonemertes palaensis.* Portion of transverse section of body, showing hermaphroditic gonad (*h*) with spermatids and two small ovocytes (*oc*), one small ovary (*ov*) with ovocyte and follicle cells, also two undifferentiated gonads (*und*). Other letters as in Figure 5. Scale beneath drawing shows magnification.

likely to be found by collectors. In *G. dendyi,* for example, no male or male-phase individual has yet been reported although females have been found by Dakin (1915) in the natural habit of the species, in Aus-

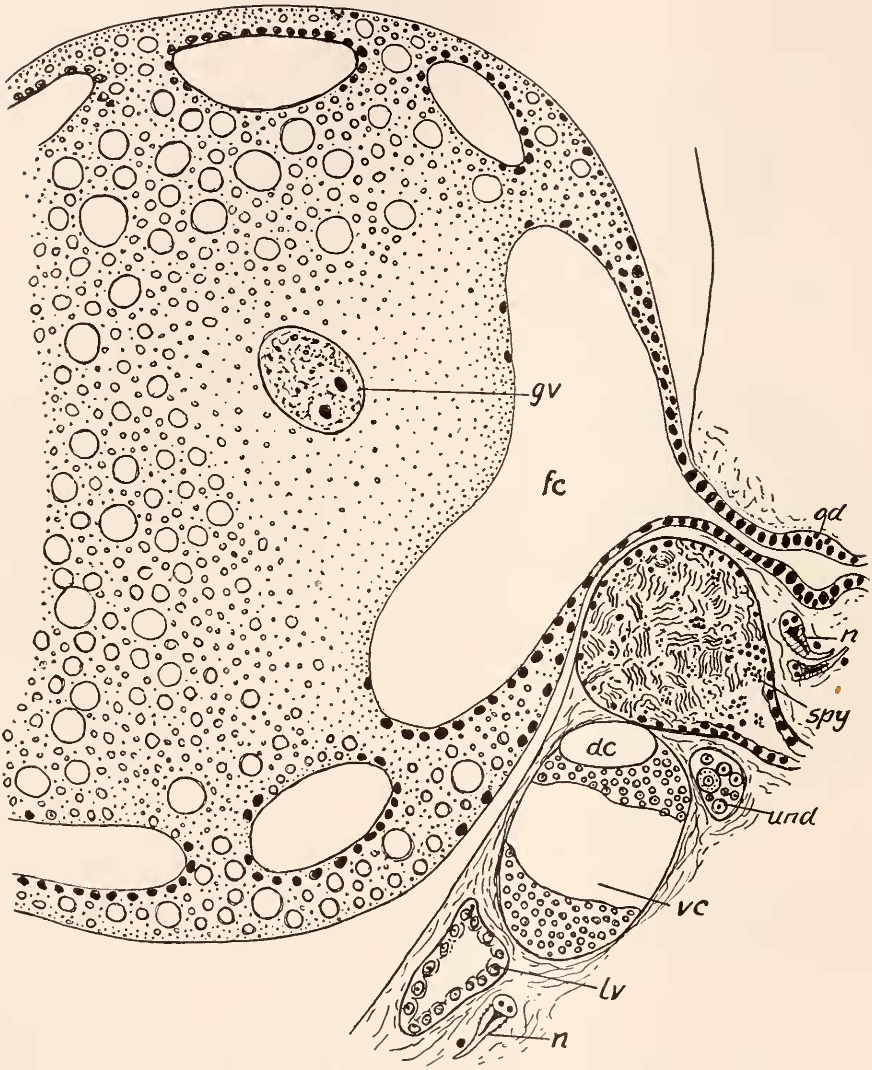

FIG. 5. *Geonemertes palaensis.* Portion of transverse section of body, showing fully ripe spermary (*spy*) and mature ovum ready for fertilization, also a small, undifferentiated gonad (*und*). Other letters indicate: *fc,* portion of original ovarian cavity, now converted into the fertilization chamber continuous with the oviduct (*od*); *gv,* germinal vesicle; *dc* and *vc,* dorsal and ventral cores of lateral nerve cord; *lv,* lateral blood vessel; *n,* flame cells of nephridial system. Same magnification as Fig. 4.

tralia, and by Stammer (1934) in Germany and Waterston and Quick (1937) in Wales, into which countries it has been introduced. Stammer has suggested the possibility that all the terrestrial species are protandric hermaphrodites. Such proves to be the condition in the two species which form the basis of the present paper but the extent to which the suggestion may apply to other species can be determined only after their life histories have been more completely investigated.

Ovogenesis

The young ovary usually has a single ovocyte on the wall nearest the center of the body, with a number of small follicle cells lining the ovarian cavity (Figs. 4, 5). As the ovocyte increases in size it becomes partially separated from the ovarian wall by wide spaces continuous with the ovarian cavity, eventually remaining attached to the wall by broad pseudopodia only. These pseudopodia then spread out and flow together distally to form a continuous cytoplasmic lining on the ovarian wall. This peripheral cytoplasm becomes widely separated from the main body of the ovocyte by these extensions of the ovarian cavity but remains united with it by large protoplasmic connections (Fig. 5). The surface of the ovum is thereby greatly increased.

Meanwhile the follicle cells have multiplied rapidly and spread as a continuous layer of tissue covering the peripheral cytoplasm and now function in the formation of yolk. As rapidly as the yolk is formed it is carried by cytoplasmic streaming into the main portion of the rapidly growing ovocyte. With the completion of yolk-formation, the peripheral cytoplasm is drawn into the ovocyte which then assumes a nearly spherical form.

Before the egg is mature the follicle cells lining the walls of the distal ovarian chamber form a continuous, epithelial-like layer which grows distally through the connective tissue and muscular layers of the body wall to form the oviduct (Figs. 4, 5). When the ovum is fully ripe the oviduct is completed through the surface epithelium of the body and provides an opening through which sperm may enter and through which, in the oviparous species, the egg will be discharged later. The remaining ovarian cavity provides a fertilization chamber through which the sperm passes before entering the yolk-free animal pole of the ovum (Fig. 5). The presence of two mature ova in a single ovary is very unusual in *G. palaensis* but occurs occasionally in *G. agricola*. In the young ovaries, however, two or more ova are of common occurrence (Fig. 4) but all except one of these are ingested or cytolysed during ovogenesis. The yolk, however, is formed more largely by the activities of the follicle cells than by the ingestion of the smaller ovocytes.

General Discussion

Development of Gonads

In the hermaphroditic species three types of gonads are often present in the same individual: (*a*) spermaries, (*b*) hermaphroditic gonads which have retained their early ambisexual character and contain both sperm-forming cells and immature ovocytes, and (*c*) ovaries. The relative proportion of each of these three types at each sexual phase is highly variable. Except in young individuals the ovaries are much more numerous than either of the other types. Indeed, there are often several hundred times as many ovaries as spermaries and in some cases the worm passes through a strictly female phase. In other individuals there may be a few spermaries at either end of the body or they may be scattered between the ovaries. It must be remembered, however, that this condition applies to the individual at the time of observation or the time of collection and does not indicate the sexuality which has obtained at an earlier period of life or might have followed later if the animal had survived.

The hermaphroditic gonad may occasionally function first as a spermary and later as an ovary but more often the ovocytes present undergo cytolysis before the sperm are fully mature. Otherwise each gonad functions but once, its place being taken by a younger one following the discharge of the gametes, or of the embryo in the viviparous species, as *G. agricola*. New gonads are formed throughout life from groups of germinal cells situated along the dorsal borders of the nerve cords (Figs. 4, 5). It is conceivable that the sexual differentiation of the gonad may depend upon the nutritive conditions of that region of the body at the time of differentiation. Presumably when closely crowded or when originating in an unusual position on the distal side of the nerve cord, as well as when rapidly growing ovocytes or embryos are present nearby, the gonad becomes differentiated into a spermary. When a spermary develops in the same interdiverticular space as an ovary the former usually lies nearest the nerve cord.

Fertilization

In nearly all the species in which living individuals have been available for study the worms have been observed to collect together in groups with the bodies of two or more individuals placed side by side, accompanied by the secretion of considerable mucus. If two or more of these individuals are provided with ripe sexual products when thus in contact the sperm cells from one individual may be readily transferred to the fertilization chambers of another which may contain mature ova and open oviducts. Internal insemination occurs in all terrestrial species so far as known but is not confined to that group, since it is also characteristic of the fresh-water nemerteans and some of the marine species.

In *G. australiensis,* in which the sexes are separate, such copulation and the transfer of sperm from the small male to the larger female has been observed (Dendy, 1893). The sperm thus transferred to the ovaries may remain functional for several days or perhaps weeks, ready to enter the egg in each ovary as soon as it has become sufficiently mature. Although the male in *C. dendyi* has not yet been discovered, Waterston and Quick (1937) reported that several of the females collected by them laid clusters of fertilized eggs a few days after capture.

In the hermaphroditic species the young, male-phase individual presumably may thus transfer his sperm to either an individual functioning at the time as a female or to an hermaphrodite with both ripe eggs and sperm; likewise two hermaphrodites may transfer sperm mutually or an hermaphrodite may inseminate a functional female. It seems quite possible that in some cases individuals of all these sexual phases may be grouped together at the same time. Microscopic sections sometimes show the presence of several spermatozoa in a fertilization chamber.

In terrestrial nemerteans insemination thus precedes ovulation if two individuals with suitable sexual products come in contact and this is the case with a few marine and fresh-water forms. In the great majority of nemerteans, however, the eggs are fertilized at the time of ovulation or in some cases, as in *Cerebratulus,* vast numbers of minute ova are set free in the water with the possibility of fertlization by sperm originating from a male which may be at least several meters distant.

There appears to be no fixed limit to the number of sexual phases which an individual may experience during its lifetime, since even in the largest worms bearing ripe ova or embryos, according to the species, there are always present many newly-formed, undifferentiated gonads in the parenchyma adjacent to the dorsal sides of the nerve cords (Figs. 1, 4). Recuperation periods, some of which may be of a seasonal nature, may, presumably, long delay the completion of any of these phases.

Self-fertilization

In the hermaphroditic species, as *G. agricola* and *G. palaensis,* spermaries with fully functional sperm are frequently present in some part of the body and sometimes immediately adjacent at the time when the ova are ready for fertilization. It would appear that in isolated individuals the conditions are highly favorable for self-fertilization, although direct evidence of such an occurrence is not available. It is well-known, however, that in some hermaphroditic nemerteans, as the fresh-water *Prostoma,* self-fertilization takes place frequently.

It is remarkable that among the 12 known species of terrestrial nemerteans, all of which belong to a single genus, there should be so much similarity in morphological and ecological characteristics and so great a diversity in the physiology of reproduction. Some of the species are hermaphroditic and others presumably of separate sexes; most of them are known or believed to be oviparous but at least one species is regularly viviparous.

Viviparity, protandry and hermaphroditism occur also in a few species of marine nemerteans belonging to different groups, and in some species of *Prostoma, Carcinonemertes* and *Prosorhochmus* which are normally oviparous, with internal fertilization, a few of the fertilized ova may be retained in the mother's body until the embryo is far advanced. Since in *Geonemertes* the same type of fertilization exists and at least one of the species is regularly viviparous it seems quite possible that occasional viviparity will later be found in some of the little-known species now thought to be exclusively oviparous.

Summary

1. A series of 21 specimens of a species of terrestrial nemertean, *Geonemertes palaensis,* from tropical Pacific islands includes successive stages in the development of the gonads which show that this species is normally protandric and irregularly hermaphroditic, as previously described for *G. agricola.*

2. The primary male phase of the young individual is followed by a female phase which, however, may become functionally hermaphroditic in some individuals before the ova are fully ripe by the precocious development of some of the spermaries of the normally succeeding male phase. Opportunity is thereby offered for self-fertilization.

3. The extent to which the female phase toward its close is accompanied by the development of spermaries is highly variable, some individuals having in this phase only a few small spermaries and others many larger ones.

4. This sequence of alternating male and female (or hermaphroditic) sexual phases presumably continues throughout the lifetime of the individual.

5. *Geonemertes palaensis* is oviparous, while the terrestrial nemertean of Bermuda (*G. agricola*) is viviparous. The sexual phases, however, are similar in both species.

6. No direct evidence is available as to whether the great variability in the relative proportions of male and female gonads in different indi-

viduals following the initial male phase in both these species is due to physiological conditions imposed by environmental influences or to slight differences in the hereditary sexual endowment. Presumably both these factors may be associated.

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